(19) [logo] European Patent Office (11) Publicn. **No. 0 421 021 A1**

EUROPEAN PATENT APPLICATION

(21) Appl. No.: **89118625.6** (51) Int. Cl[5]. **A 61 K 37/54**

(22) Appl. date: **10/06/1989**

(43) Date of publicn. of applicn:
04/10/1991 Patent Bulletin 91/15

(84) Designated contracting states:
AT BE CH DE ES FR GB GR IT LU NL SE

(71) Applicant: **MUCOS EMULSIONSGESELLSCHAFT mbH**
Miraustrasse 17
W-1000 Berlin 27(DE)

(72) Inventor: **Ransberger, Karl**
Gabriel-von-Seidl-Strasse 62, W-8022 Grunwald (DE)
Inventor: **Stauder, Gerhard**
Kathi-Kobus-Steig 1
W-8190 Wolfratshausen (DE)

(74) Representative: **Patent Attorneys Grunecker, Kinkeldey, Stockmair & Partner**
Maximilianstrasse 58
W-8000 Munich 22 (DE)

Use of catabolic enzymes for the activation of macrophages and/or NK cells, a medication containing this enzyme, and macrophages and/or NK cells activated by this enzyme.

(57) The invention relates to the use of one of the enzymes pancreatin, bromelain, papain, lipase, amylase, trypsin and/or chymotrypsin for the activation of macrophages and/or NK cells, enzymatically activate macrophages and/or NK cells, as well as a medication for the activation of macrophages and/or NK cells.

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USE OF CATABOLIC ENZYMES FOR THE ACTIVATION OF MACROPHAGES AND/OR NK CELLS, A MEDICATION CONTAINING THIS ENZYME, AND MACROPHAGES AND/OR NK CELLS ACTIVATED BY THIS ENZYME.

The present invention relates to the use of the enzymes pancreatin, bromelain, papain, lipase, amylase, trypsin and/or chymotrypsin for the activation of macrophages and/or NK cells, enzymatically activated macrophages and/or NK cells, as well as a medication for the activation of macrophages and/or NK cells.

Designated as macrophages are mononuclear phagocytes (monocytes), which belong to the phagocytosing leucocytes.

NK ("natural killer") cells represent a sub-population of the lymphocytes.

Macrophages and NK cells have an important function in the immunological surveillance both of tumor cells and also of infections. Thus it has long been known that activated macrophages and NK cells can destroy a whole series of different tumor cells in non-specific ways in vitro and in vivo. The activation of the tumoricidal effector cells (macrophages, NK cells), can be raised with lymphokines such as interferon and other immunostimulants. Using large scale and expensive methods, interferons are either isolated from cell cultures or synthesized by means of genetic engineering methods.

The present invention is based on the problem of providing an economical, effective activator of the tumoricidal potential of effector cells (macrophages, NK cells), as well as enzymatically activated effector cells and a medication for the activation of effector cells.

This problem is solved in accordance with the invention by the fact that at least one of the enzymes pancreatin, bromelain, papain, lipase, amylase, trypsin and/or chymotrypsin is used for the activation of macrophages and/or NK cells.

It has surprisingly been shown that the enzymes pancreatin, bromelain, papain, lipase, amylase, trypsin and/or chymotrypsin can activate macrophages and/or NK cells highly effectively and in an extremely short time (within 5-10 minutes).

The enzymes used in accordance with the invention can be isolated economically from the following raw materials.

Pancreatin is obtained from pig or cattle pancreas.

Bromelain is a proteolytically active enzyme from the pressed juice of the pineapple.

Papain is a proteolytic enzyme that is obtained from the milky juice of the unripe, fleshy fruits of the pawpaw, Carica papaya.

Lipases belong to an esterase sub-group and are obtained from the pancreas or the fungus Rhizopus arrhizus.

Amylases are glycoside-splitting enzymes that are for example isolated from the pancreas or specific microorganisms.

Trypsin and chymotrypsin are proteolytic enzymes that are also formed in the pancreas and have already been used therapeutically in combination with other enzymes.

Triacylglycerol lipases are preferably used as the lipases, and/or the amylase is α-amylase. These display good activity as activators of the tumoricidal potential of macrophages and/or NK cells.

A particular efficacy is shown when a combination of the enzymes pancreatin, bromelain, papain, triacylglycerol lipase, α-amylase, trypsin and/or chymotrypsin is used. Besides the remarkable and unexpected effect of these enzymes on the activation of the tumoricidal potential of macrophages and/or NK cells, the use in combination of the enzymes cited also shows a synergistic effect.

Furthermore, rutoside, a glycoside belonging to the flavonoids, can preferably be used in addition.

The use in combination of 50-200 mg, preferably 100 mg pancreatin, 20-100 mg, preferably 45 mg bromelain, 40-100 mg, preferably 60 mg papain, 5-50 mg, preferably 10 mg triacylglycerol lipase, 5-50 mg, preferably 10 mg α-amylase, 10-30 mg, preferably 24 mg trypsin, 1-10 mg, preferably 1 mg chymotrypsin and 10-100 mg, preferably 50 mg rutoside.$3H_20$ per dose unit has a particularly good tolerance and efficacy.

Further, the preparations to be used can in addition contain Serratia peptidase. Serratia peptidase can be obtained from a microorganism of the Serratia genus.

In a particularly preferred form of execution, polyethylene glycol (PEG) and/or polyvinylpyrrolidone (PVP) are used in addition. In this way, the activity of effector cells can be additionally raised.

The preparation utilized can also contain the usual adjuvants and/or carriers.

The enzymatically activated macrophages and/or NK cells obtained by treating the effector cells with at least one of the enzymes pancreatin, bromelain, papain, lipase, amylase, trypsin and/or chymotrypsin can be injected into the patient for the treatment of tumors. It has surprisingly been demonstrated that the enzymatically activated state of the effector cells is preserved after freezing and later thawing. It is of advantage here if the enzymatically treated effector cells can regenerate for at least 20 minutes, preferably at least 30 minutes, before freezing or injection.

The examples illustrate the invention.

I. Activation of macrophages

In the study of the effect of enzymes and enzyme mixtures on the activity of macrophages, the following parameters were determined:

a) (PR%) Relative phagocytosis rate (in %) designates the phagocytosed percentage fraction of the target cells, i.e. of the ^{51}Cr labeled sheep erythrocytes (SRBC), present in the selected standardized test arrangement of macrophages/monocytes.

The calculation in the ^{51}Cr phagocytosis test - in far-reaching analogy to the calculation of the specific lysis in the ^{51}Cr-release cytotoxicity test - is carried out using the formula:

Relative phagocytosis rate (in %)

$$\frac{\text{cpm (sample) - cpm (negative control)}}{\text{cpm (total) - cpm (blank)}}$$

b) (dPR %) Relative increase in the phagocytosis rate (in %) designates the percentage rise in the phagocytosis rate due to enzymatically pretreated monocytes/macrophages relative to untreated monocytes/macrophages.

c) (PI) Phagocytosis index designates the phagocytized fraction of target cells (^{51}Cr-labeled SRBC) per 100 monocytes/macrophages in a sample (well in the 96-well microtitration plate).

For the calculation of the phagocytosis index, in addition to the phagocytosis rate, the number of SRBC in a sample well and the number of monocytes/macrophages in this well, or at least the ratio of SRBC to monocytes/macrophages, i.e. of target (T-) and Effector (E-) cells must be known. Then it holds that:

$$PI = T/E \times PR\ \% \times 100 \qquad \frac{\text{number of SRBC per well}}{\text{number of macrophages per well}} \times PR\ \% \times 100$$

d) (dPI) Change in the phagocytosis index designates the number of SRBC in a sample that are phagocytized more (or less) per 100 monocytes on the average, if the monocytes were pretreated with enzymes, measured as that number of SRBC phagocytized by 100 monocytes in the base medium (without enzyme).

$$dPI = PI\ (\text{pretreated}) - PI\ (\text{untreated})$$

e) (f) phagocytosis change factor designates the quotient of the phagocytosis rates of pretreated and untreated samples.

$$f = \frac{PR\ \%\ (\text{pretreated})}{PR\ \%\ (\text{untreated})} = \frac{\text{cpm (pretreated) - cpm (negative control)}}{\text{cpm (untreated) - cpm (negative control)}}$$

Activation of macrophages by an enzyme mixture (Wobenzyme)

Wobenzyme is an enzyme mixture with the following composition (parts by weight): pancreatin (100), bromelain (45), papain (60), triacylglycerol lipase (10), α-amylase (10), trypsin (24) and chymotrypsin (1). In addition, the enzyme mixture also contains 50 parts by weight rutoside.$3H_20$.

a) Tenfold plasma concentration ($10 \times C_{PI}$)

Test series I (test conditions: recovery time: 30 min, enzyme action duration: 10 min, pre-centrifuging (PC): 3 min/50 g, E:T ratio: 1:1-20).

The average PR % value for samples without enzyme treatment was 10.3 and for samples with enzymatic pretreatment 14.7. The corresponding PI values were 92.5 and 129.4. Thus the macrophage pretreatment with Wobenzyme brought an average improvement in the PI value of 36.9. The f value was 2.21.

Test series II (test conditions as for test series I, except E:T = 1:20-40).

Here, the PR % value of untreated samples was 2.8 and that of the enzyme pretreated samples 7.6. Corresponding PI values were 95.2 and 258.4. Correspondingly, the dPI value was raised by 163.2. The f value was 2.75.

Test Series III (Test conditions as in test series I, but E:T = 1:40).

The untreated samples displayed a PR % factor of 2.4, the enzyme-treated samples one of 4.6, and the corresponding PI values were calculated at 161.7 and 282.9. Thus the Wobenzyme treatment brought an increase in the dPI of 121.2. The f-value amounted to 1.75 on the average.

b) Single plasma concentration (C_{PI})

Test series I (Test conditions: recovery time: 30 min, enzyme action duration: 10 min, precentrifuging (PC): 5 min/50 g - 3 min/80 g, E:T ratio: 1:1 - 1:40, tz.v = 90 [meaning of tz.v unknown- Translator]).

The PR % value after repeating the same test 6 times was on the average 4.4 compared with 2.5 in the absence of enzymes. The corresponding PI values were 138.8 and 111.3. Thus the pre-treatment of the macrophages with Wobenzyme led to an average rise in the dPI of 27.5.

Test series II (Test conditions: as in test series I, but tz.v = 150).

The tests, repeated 7 times, gave for the enzyme pretreatment of the macrophages an average PR % value of 13.2 and an average PI value of 390.6. The corresponding averages for untreated samples were 10.8 and 271.0. The average rise in dPI after enzyme treatment was 119.6, the f index 1.53.

Test series III (Test conditions: as in Test series I, but tz.v = 250).

The result for five individual experiments showed that Wobenzyme allowed the dPI index to rise by an average of 17.4. The PI values of untreated samples were on the average 524.5 and of enzymatically pretreated samples 541.9. The corresponding PR % values were 19.0 and 19.7.

The activity of the macrophages could be raised further if PEG and/or PVP was added to the culture medium in addition to the Wobenzyme.

Test series IV

In a test series consisting of 6 individual tests, an average dPI increase of 148.9 could be observed as a result of the rise in the PI from 134.5 to 283.4 after combined Wobenzyme/PEG-PVP pre-treatment. The PR % value after the combined Wobenzyme/PEG-PVP pretreatment rose from 3.8 to 7.9.

Test series V

After a test series consisting of 9 individual tests, in which macrophages were pretreated in part with Wobenzyme alone, in part with the combination of PEG-PVP and Wobenzyme, the PI value rose by an average of 108.7.

Test series VI

In another test series composed of 18 individual tests, the macrophage pre-treatment with proteases and lipases of the enzyme preparation Wobenzyme (C_{PI}) could effectuate an average increase in dPI of 60.2. The average PI value for the untreated samples was 288.2 and that for the enzyme-treated samples 348.4. The corresponding PR % values were 12.8 and 9.9.

The statistical processing of the PR %, PI, dPI and f values obtained in 48 individual tests with 3-4 parallels each, in a few cases 2 parallels, showed that the pre-treatment of the

monocytes/macrophages with Wobenzyme alone (C_{PI}) or with the combination of Wobenzyme (C_{PI}) and PEG-PVP (2-20 g/l PEG of MW 6000 D and 1-10 g/l PVP of MW 40,000 D and 1×10^{-3} - 1×10^{-4} g/l PVP of MW 360,000 D) leads to an average increase in PR % of 8.4 (for untreated samples) to 11.5 (after macrophage treatment). The corresponding average PI values were 238.1 (for untreated samples) and 319.8 (for pretreated samples). The dPI values were on the average 81.5, and the f value was 1.90.

2. The efficacy of hydrolases (Wobenzyme) used in combination with PEG and PVP

In 2 parallel test series in each case, (test series XI-XIV), under otherwise constant test conditions, the efficacy of the macrophage pre-treatment with Wobenzyme alone (C_{PI}) was compared with that after combined, i.e. PEG-PVP-assisted, enzymatic pre-treatment of macrophages.

The first two parallel test series (test series XI and XII) differed from the other two (test series XIII and XIV) only in that tz.v in the former case was 150 and in the latter case 250. The other test conditions were constant (E-T precentrifuging: 5 min/50 g, regeneration time: 30 min).

A direct comparison of the PR %, PI and dPI values between test series XI and XII (tz.v = 150) as well as between test series XIII and XIV (tz.v = 250) conveyed a picture of the potentiating effect of PEG and PVP on the activation of monocytes/macrophages by hydrolases (Wobenzyme).

Thus the average PI value rose for treatment with Wobenzyme alone from 271.0 (untreated) to 390.6 (treated), corresponding to a dPI value of 119.6, while the corresponding PI value for combined macrophage pre-treatment "jumped" from 224.4 to 517.0, which corresponds to a dPI value of 292.6.

The PR % value increased for treatment with Wobenzyme alone from 10.8 (untreated) to 15.2 (treated); for combined pre-treatment it changed from 5.1 to 11.7.

These results are based on tz.v = 150 (test series XI and XII). With tz.v = 250 (test series XIII and XIV), the comparison between single and combined Wobenzyme pre-treatment proved to be as follows:

The average PI value for treatment with Wobenzyme alone rose [sic] from 524.5 (untreated) to 514.9 (treated), corresponding to a dPI value of 17.3 [sic]. For the pre-treatment combined with PEG and PVP on the other hand, the dPI value rose by 134.8 units (from 173.2 to 308.1). The PR % values in the absence of PEG and PVP rose from 19.0 to 19.7, and in their presence from 6.4 to 11.5.

3. Activation of the macrophages by trypsin (C_{PI})

The test conditions corresponded to those with Wobenzyme (recovery time: 30 min, enzyme action time: 10 min, pre-centrifuging: 3 min /50 g). The main variation was in the ratio between effector cells (macrophages) and target cells (^{51}Cr-labeled SRBC), i.e. the E:T ratio.

4. E:T ratio: 1:1-10

The PR % value, i.e. the "relative phagocytosis rate" (in %) was changed on the average from 11.1 to 13.4 as a result of the pre-treatment of the macrophages with trypsin (C_{PI}).

The PI value, i.e. the total phagocytosed number of SRBC per 100 monocytes in a sample, rose in the same time from 92.5 to 111.9. The dPI value thus rose by 19.5 units. The f factor, corresponding to the quotient of the phagocytosis rates of the treated and untreated samples, was 1.43.

E:T ratio 1:10-20

Trypsin treatment of the macrophages led to a rise in the "relative phagocytosis rate" (PR %) from 17.9 to 24.2 and to an increase in the phagocytosis index (PI) from 233.3 (untreated) to 316.1 (treated).

The dPI value showed an average value of 82.8 and the phagocytosis change factor f showed an average value of 1.32.

E:T ratio: 1:40-80

This complex including 7 individual tests with 26 parallels showed the following trends: PR % rose from 4.76 to 5.3 and PI from 204.9 to 225.5. The phagocytosis index changed by 20.6 and the f factor showed a value of 1.24 on the average.

E:T ratio: 1:80

The test, carried out 4 times (with 15 parallels), showed a similar trend to the test series above: PR % rose moderately from 3.1 to 3.7 after trypsin treatment. In the same time, the phagocytosis index was raised from 275.1 (without pre-treatment) to 325.5. The f factor was 1.19, the average dPI index 50.4.

E:T ratio: 1:1-80

The combination of 18 tests (with 68 parallels) without more detailed specification of the E:T ratios resulted in a rise in the average PR % value from 8.0 (before treatment) to 9.9 (after treatment), a PI increase from 200.3 to 237.6 and an average value for dPI of 37.2 and for f of 1.28.

5. Observance of a required regeneration time of at least 20, preferably 30 minutes in the in vitro activation of macrophages by hydrolases, demonstrated in the example of trypsin (C_{PI})

In the following test, the observance or nonobservance of a regeneration time of 30 min after enzyme treatment was investigated. If no regeneration time was observed, then the average "relative phagocytosis rate" (PR %) fell from 8.3 to 7.9. Also, the averaged PI decreased from 252.7 to 219.2. Thus the dPI was negative (-33.5) and the f factor was 0.86. When however, under test conditions that were otherwise the same, a regeneration time of 30 min was observed, there was on the contrary a change in the PR % after trypsin treatment from 7.9 to 9.9, the PI went from 200.2 to 237.6, the dPI displayed an average value of 37.3 and the f factor was 1.28.

6. Macrophage activation by papain

Two papain concentrations were tested, the C_{PI} and ten times that concentration. In the first test series, papain was investigated in the C_{PI} concentration. The papain-pretreated macrophages displayed a PR % average value of 11.3 and an average PI value of 165.9. The untreated macrophages, on the other hand, displayed a lower PR % average value of 7.5 and an average PI value of 164.6.

The tenfold higher concentration of papain effectuated a PR % rise from 2.9 to 5.4 and a PI shift from 139.0 to 265.3. The average dPI value was 196.2 [sic] and the f average 1.85.

7. Activation of the effector cells (macrophages) by lipase

The test conditions were the same as for the other enzymes. In a test series consisting of 8 individual tests (with 29 parallels), the pre-treatment of the macrophages with the therapeutically obtainable lipase concentration (C_{PI}) resulted in a rise in the phagocytic activity of the macrophages that was expressed in the following (averaged) indexes: the relative phagocytosis rate (PR %) rose from 10.3 for untreated samples to 12.3 for samples pretreated with lipase. The phagocytosis index (PI) was raised from 258.8 to 316.8 units. The dPI average value was calculated at 58.0 and the f average was 1.24 on the average.

In another series of 8 individual tests, in which the lipase was sometimes also combined with PEG (MW 6000 D, 40 g/l) and PVP (MW 360,000 D, 0.1 - 10 mg/l), the relative phagocytosis rate after enzyme treatment was raised from 16.6 to 20.4 and the phagocytosis index from 329.9 to 411.2. The corresponding dPI average value was 59.0 [sic] and the averaged f value 1.24.

The combined representation of 26 individual tests (with 76 parallels) gave on the average a PR % rise from 10.1 to 12.8 (in the extreme case from 3.0 to 13.2) and an average PI increase from 230.1 to 296.3 (in the extreme case from 75.0 to 330.0). The dPI average value was 66.3 (maximum value: 255.0) and the f average 1.35 (maximum value: 4.37).

8. Synergism of the individual hydrolases (proteases and lipases)

Surprisingly, a synergistic effect of the hydrolase mixture Wobenzyme was found. Below, the effect of individual representatives of the animal and plant proteases as well as lipase was contrasted with the effect of the enzyme preparation.

a) Comparison of trypsin (C_{PI}) with the enzyme preparation (C_{PI})

In a test series encompassing 13 individual tests, it was shown that the average PR % value for Wobenzyme (10.2) was higher by 1.8 than for trypsin (8.4), and their ratio was 1.2.

The PI index for Wobenzyme (274.0) was 49.7 units higher than for trypsin (224.3). In the extreme case, the PR % values differed by 8.1 and the PI index by 281.6 units in favor of the Wobenzyme.

b) Comparison of chymotrypsin with the enzyme mixture

A test series based on 9 individual tests led to the following result:

The average value of the "relative phagocytosis rate" for Wobenzyme (12.2) was 2.4 units higher than for chymotrypsin (9.8); the "phagocytosis index" for Wobenzyme (264.0) showed a 24.8 units higher average value than for chymotrypsin (239.2). The ratio of the two PR % values was 1.26 in favor of the Wobenzyme.

In the extreme case, the PR % values differed by 13.1 and the PI values by 170.3 in favor of the enzyme mixture.

c) Comparison of papain with the enzyme mixture

In this comparison, also, Wobenzyme displayed a higher PR % activity than papain (19,5 versus 14.8).

The PI values were also higher for Wobenzyme (311.2 versus 269.0).

d) Comparison of lipase with the enzyme mixture

In a test series encompassing 5 individual tests, Wobenzyme, with a PR % average value of 11.5 displayed a 2.5-unit higher phagocytosis-raising activity than lipase (9.0). The same holds for the average PI values (292.3 versus 275.6).

The simultaneous investigation of (a) trypsin, (b) chymotrypsin, and (c) Wobenzyme (all: C_{PI}) in a test series consisting of 9 individual tests resulted in the following averages for the activity-relevant indexes PR % , PI and f:

(a) trypsin: PR % 10.2, PI 215.1 and f 1.37
(b) chymotrypsin: PR % 9.9, PI 239.2, and f 1.46, and
(c) Wobenzyme: PR % 12.2, PI 264.0 and f 1.74.

The corresponding average PR % and PI values of the untreated reference samples were 7.9 and 171.9 (f 1.0).

II. Increasing the activity of NK cells by hydrolases (proteolytic enzymes of animal and plant origin, lipase)

The cytotoxic/cytolytic activity of NK cells was determined quantitatively in the ^{51}Cr release test. K562 lymphoma cells served as target cells (T); they are particularly sensitive and to some extent selective for (activated) NK cells.

The parameters measured are illustrated below:

a) (LR %) Lysis rate designates mathematically the percentage cytotoxicity and is calculated by the following formula:

$$LR = \frac{\text{cpm (sample) - cpm (negative control)}}{\text{cpm (total - cpm (negative control)}} \times 100\ (\%)$$

b) (LI %) Lysis index designates the number of K562 cells in % that are lysed by 100 NK cells in each case.

$$LI = \frac{\text{number of K562* - cells (sample)}}{\text{number of NK cells (sample)}} \times \text{lysis rate (in \%)} \times 100$$

[* typo in original]

c) (dLR) Change in lysis rate designates the difference in the lysis rates (LR) between enzyme-treated and untreated samples. The reference value is thus the NK activity in the base medium, i.e. without enzyme and/or addition of PEG-PVP.

1. <u>NK activation with observance of a regeneration time</u>

The pretreatment of NK cells (10 min at 37°C) with the enzyme mixture (Wobenzyme) at the therapeutically obtainable plasma concentration (C_{PI}) showed in different test subjects greatly differing absolute values for the "lysis rate" (LR), for the lysis index (LI) as well as for the change in lysis rate after enzyme treatment (dLR); the trend to enzyme-mediated NK activation is however clearly marked.

In a test series consisting of 26 individual tests, only in one case was a decrease in activity shown after Wobenzyme treatment.

In order to convey a more precise picture, above all with regard to the considerable inter-individual deviations, the LR, LI and dLR values of this representative test series are specified separately. Thus the LR value changed from test subject to test subject corresponding to the pairs of numbers to the left and right of the slash (left: untreated samples, right: enzyme pretreated samples):3.0/3.5; 3.0/4.9;3.1/20.0; 3.1/47.4; 6.2/8.0; 6.2/8.9; 12.3/13.4; 12.3/36.1; 14.1/16.5; 14.1/42.4; 2.9/11.0; 8.5/12.1; 10.4/19.3; 20.8/21.3; 30.2/44.6; 25.2/26.5; 0.9/12.6; 5.7/31.3; 7.8/25.2; 18.4/25.5; 25.7/37.0; 25.1/25.5; 19.2/17.7; 22.1/23.6; 23.7/24.1; 23.1/24.3.

The corresponding LI values changed from test subject to test subject as follows: (left: untreated, right: enzyme pretreated): 60/71; 60/98; 62/401; 62/948; 124/160; 124/178; 246/267; 246/721; 282/330; 282/849; 57/219; 170/241; 207/387; 416/425; 603/892; 503/530; 17/252; 115/626; 157/505; 368/511; 514/739; 502/513; 384/354; 442/473; 474/482; 462/485.

The dLI values [sic] calculated from this were: 11; 38; 339; 886; 36; 54; 21; 475; 48; 567; 162; 71; 180; 9; 289; 27; 235; 511; 348; 143; 225; 11; 31; 8; 23.

2. Treatment of the NK cells with the enzyme mixture Wobenzyme without observance of a regeneration time

The values become mostly negative in these studies. LR varied from 0.7/3.3-18.7/7.2, the values for LI were between 14/67 and 431/233, and for the dLI between 53 and -229. The results make clear the importance of a regeneration time for the cells.

3. Activation of the NK cells with trypsin

Trypsin proves to be one of the most active components of the hydrolase mixture. In order to show the clear effect, but also the great inter-individual deviations that involve both the base activity and also the enzyme-mediated activatability of the NK cells, the results of a test series consisting of 28 individual tests are listed separately. As above with Wobenzyme, the LR, LI and dLI values are reproduced. Here, too, the number to the left of the slash stands for the untreated samples and that to the right of this slash for the enzyme pretreated samples.

The LR values were: 7.6/12.8; 3.0/3.6; 7.6/18.8; 3.0/7.6; 3.1/32.6; 6.2/12.4; 3.1/32.6; 6.2/12.4; 12.3/25.9; 6.2/12.9; 12.3/26.4; 14.1/36.3; 17.3/17.8; 14.1/37.6; 25.4/27.1; 21.3/22.9; 22.8/23.8; 25.3/27.8; 22.6/26.6; 42.2/26.6; 30.3/31.9; 13.0/17.5; 20.0/24.1; 20.6/26.4; 24.5/21.4; 30.4/32.3; 23.3/27.1; 27.5/30.9; 26.6/31.8.

The corresponding LI values varied from test subject to test subject as follows: 155/256; 60/72; 155/377; 60/152; 62/651; 124/249; 62/239; 124/259; 246/517; 282/727; 246/538; 282/752; 345/355; 508/542; 425/458; 455/476; 505/556; 452/533; 483/533; 605/637; 260/350; 404/483; 413/527; 490/429; 608/646; 456/541; 549/619; 532/635.

The dLI values had the following values: 101; 12; 222; 92; 589; 125; 177; 135; 271; 445; 292; 470; 10; 34; 33; 21; 51; 81; 50; 32; 90; 79; 114; 61; 38; 76; 70; 70; 103.

4. Effect of chymotrypsin on the activity of NK cells

With chymotrypsin, too, the same effect was shown as with Wobenzyme and trypsin. An increase in the NK activity was always shown, as long as the required recovery time was observed directly after the enzyme treatment.

In a typical experiment, based on 6 individual tests, the pairs of values for the "lysis rate" (LR) varied between 6.2/9.2 and 3.1/17.5, those for the "lysis index*" (LI*) between 124/185 and 62/349, and for the dLI* values between 61 and 287.

[* typos in original]

5. Increasing the NK activity with papain

A clear increase in activity was also shown with NK cells that had been pretreated with the plant protease papain.

This is demonstrated in the example of a representative test series based on 8 individual tests.

The LR value pairs varied from test subject to test subject from 0.7/1.9 to 14.1/45.7, the corresponding LI value pairs were between 14/37 and 282/915, and the dLI values were scattered between 23 and 633.

6. Raising the activity of NK cells with lipase

The influence of the lipase contained in the Wobenzyme on the NK activity is demonstrated in the example of a test series consisting of 32 individual tests. It was shown that there is a clear effect on the activity of NK cells, but that the inter-individual deviations both in the base activity and also in the lipase-mediated activation of NK cells are significant. Only in 3 of 32 cases were there negative values for the dLI.

The subdivision according to the activation indexes LR, LI and dLI, as well as the way of writing the LR and LI pairs of values (left: untreated, right: pretreated with lipase), correspond to those for the enzymes discussed above.

LR ("lysis rate"): 2.2/3.1; 5.7/7.1; 4.6/7.1; 4.3/5.5; 2.4/7.3; 5.9/7.2; 7.1/7.2; 5.7/6.4; 4.3/9.6; 1.9/1.4; 5.2/6.4; 3.4/5.4; 4.7/5.2; 4.2/7.0; 5.8/.5; 6.5/6.5; 6.1/7.5; 7.0/11.0; 19.2/17.0; 22.1/23.6; 23.7/24.1; 23.1/23.4; 6.2/8.4; 6.2/7.1; 12.3/26.3; 12.3/26.0; 14.1/38.2; 14.1/39.7; 14.7/15.1; 18.7/19.4; 21.6/13.9; 19.6/20.1.

LI ("lysis index"): 43/62; 115/142; 93/142; 86/109; 48/147; 118/145; 143/144; 114/128; 86/191; 38/29; 105/128; 68/109; 94/104; 83/139; 116/150; 129/131; 150/122; 140/221; 384/340; 442/471; 474/481; 462/469; 124/167; 124/142; 246/527; 246/521; 282/763; 282/793; 301/293; 373/387; 431/278; 401/393.

dLI [sic] ("change in rate of lysis" [sic]): 19; 27; 49; 23; 99; 27; 1; 14; 105; -9; 23; 41; 10; 56; 34; 2; 28; 81; -44; 29; 7; 43; 18; 281; 275; 481; 511; 8; 14; -153; 8.

III. For the injection into the tumor patient of the enzymatically activated effector cells obtained in vitro, the stability of the tumoricidal state of these cells is of particular importance. It has surprisingly been found that the activated tumoricidal state of the effector cells obtained by enzymatic treatment is preserved after the freezing and later thawing of the latter.

The two polymers PEG and PVP were used as the cryoprotectants for the freezing of the enzymatically activated effector cells. In these tests, PEG of MW 6000 D and PVP with MW 40,000 D and 360,000 D in a final concentration [typo in original] of 40 g/l is added to an 8-15% DMSO solution in RPM I 1640 medium.

As can be seen from the indexes listed below, with this mixture the enzyme-induced macrophage activation is preserved even after the freezing and thawing of the macrophages.

The test conditions were: Wobenzyme (C_{PI}), Enzyme action time: 10 min, recovery time: 30 min.

The activities measured after the thawing of the frozen enzyme-pretreated macrophage suspensions clearly show that the effector cell activation is not lost by freezing the cells. Thus the samples frozen combined with the cryoprotective mixture composed of DMSO (8-15%) and PEG (MW 6000 D, 40 g/l) showed PR % values of 4.4 - 9.7, while the reference values were between 2.2 and 6.1 (PR % comparison values: 9.7/2.2, 4.4/2.6, 8.6/6.1, 6.9/2.8). The PI values varied between 132.0 and 291.0, the reference values between 66.0 and 146.4 (PI values: 291.0/66.0. 132.0/78.0, 206.4/146.4, 200.1/81.2).

The corresponding dPR values were between 41.0 and 340.7, the dPI values between 54.0 and 225.0. The good preservation of the macrophage activity was also shown in the f value which was between 1.41 and 4.41. The combination of the conventional cryoprotectant DMSO with PVP proved to be very successful in the preservation of the macrophage activity, as could also be deduced from the comparison of the PR % value pairs 5.9/2.8 and 8.7/2.1 for PVP of MW 40,000 D and 4.2/1.8 and 12.0/6.6 for PVP of MW 360,000 D.

The same result is also shown for the PI value pairs (141.6/67.2 and 60.9/252.3 for MW 40,000 D and 196.0/54.0 and 300.0/165.0 for MW 360,000 D), as well as for the f values (2.12 and 4.15 for MW 40,000 D or 2.34 and 1.80 for MW 360,000 D).

Claims

1. Use of at least one of the enzymes pancreatin, bromelain, papain, lipase, amylase, trypsin and/or chymotrypsin for the activation of macrophages and/or NK cells.

2. Use as in claim 1,
characterized by the fact that
triacylglycerol lipase is used as the lipase.

3. Use as in claim 1,
characterized by the fact that
α-amylase is used as the amylase.

4. Use as in claim 1,
characterized by the fact that
a combination of the enzymes pancreatin, bromelain, papain, triacylglycerol lipase, α-amylase, trypsin and/or chymotrypsin is used.

5. Use as in one of the claims 1-4,
characterized by the fact that
rutoside is used in addition.

6. Use as in claim 5,
characterized by the fact that
50-200 mg, preferably 100 mg pancreatin, 20-100 mg, preferably 45 mg bromelain, 40-100 mg, preferably 60 mg papain, 5-50 mg, preferably 10 mg triglycerol lipase, 5-50 mg, preferably 10 mg α-amylase, 10-30 mg, preferably 24 mg trypsin, 1-10 mg, preferably 1 mg chymotrypsin, and 10-100 mg, preferably 50 mg rutoside.$3H_20$, are used per dosage unit.

7. Use as in at least one of the claims 1-6, characterized by the fact that in addition Serratia peptidase is used.

8. Use as in at least one of the claims 1-7, characterized by the fact that in addition PEG and/or PVP is used.

9. Enzymatically activated macrophages and/or NK cells obtained by treating the cells with at least one of the enzymes pancreatin, bromelain, papain, lipase, amylase, trypsin and/or chymotrypsin.

10. Medication for the activation of macrophages and/or NK cells containing at least one of the enzymes in accordance with claims 1-8.

European Patent Office

Application number EP 89 11 8625

EUROPEAN SEARCH REPORT

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant claims	CLASSIFICATION OF THE APPLICATION (Int. Cl.⁵)
A	RED LIST, 1986, No. 85074, Wobe-Mugos --- RED LIST, 1986, No. 85075, Wobe-Mugos --- RED LIST, 1986, No. 41001, Wobenzym --- EP-A-0 309 602 (MUCOS) ---		A 61 K 37/54 TECHNICAL AREAS SEARCHED (Int.Cl.5) A 61 K
The present search report was provided for all patent claims.			

Site of search	Date of completion of search	Examiner
The HAGUE	01/30/1990	TURMO Y BLANCO, C.E.

CATEGORY OF CITED DOCUMENTS

X: Particularly relevant if taken alone
Y: Particularly relevant if combined with another document of the same category
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P: Intermediate document
T: Theory or principle underlying the invention
D: Document cited in the application
L: Document cited for other reasons
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&: Member of same patent family, Corresponding document